EXHIBIT B

30435.54USU1 SBA/RDG

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Robert Reiter and Owen Witte	
Serial No.	:	09/038,261	Examiner: Dr. Larry Helms
Filed	:	March 10, 1998	Group Art Unit: 1642
For	:	PSCA: PROSTATE STEM CELL ANTIGEN AND USES THEREOF	

35 N. Arroyo Parkway
Pasadena, California 91103

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

DECLARATION BY
ROBERT REITER AND OWEN WITTE
UNDER 37 C.F.R. §1.132

We, Robert Reiter and Owen Witte, hereby declare that:

1. The assignee of record of the subject application is the University of California at Los Angeles (UCLA), in Los Angeles, California.

2. I, Robert Reiter, began my period of employment with UCLA in 1995 and presently hold the title of Assistant Professor in the Department of Urology. Additionally, I am a Co-Director of the Prostate Cancer Program at the Jonsson Comprehensive Cancer Center. I was employed by UCLA at the time of the invention.

3. I, Owen Witte, began my period of employment at UCLA in 1980 and presently hold the title of Professor in the Department of Microbiology, Immunology, and Molecular Genetics at UCLA. In addition, in 1986, I began a joint position as Investigator of the Howard Hughes Medical Institute (HHMI) at UCLA. I was employed by the HHMI at UCLA at the time of the invention.

4. We declare that we are the inventors of the claimed inventions: (1) a method for inhibiting the growth of prostate tumor cells expressing Prostate Stem Cell Antigen (PSCA) comprising administering to a patient a monoclonal antibody designated ATCC No. HB-12612, ATCC No. HB-12616, ATCC No. HB12618, or ATCC No. HB-12617 which binds specifically to the extracellular domain of PSCA in an amount effective to inhibit growth of the prostate tumor cells (e.g., claims 44-47); and (2) a method for selectively killing a cell expressing PSCA comprising reacting a monoclonal antibody designated ATCC No. HB-12612, ATCC No. HB-12616, ATCC No. HB12618, ATCC No. HB-12617 conjugated to a therapeutic agent with the cell so that the therapeutic agent conjugated to the antibody can kill the cell (e. g., claims 48).

5. We provide post-filing confirmatory support for the methods of inhibiting the growth of prostate tumor cells expressing PSCA, as claimed in claim 44 (see Exhibits 1-13).

6. Exhibit 1 depicts experimental results showing that administering an anti-PSCA monoclonal antibody to animals injected with prostate tumor cells expressing PSCA inhibits the growth of the PSCA-expressing tumor cells (Figure 54). Exhibit 1 shows the results of mice injected with LAPC-9 cells and treated with mouse IgG, or 1G8 which is an IgG1 isotype, anti-PSCA monoclonal antibody. LAPC-9 cells are a human prostate cancer xenograft line which expresses high levels of PSCA. The mice represented in the upper panel were treated with a mouse IgG control, while the mice represented in the

lower panel were injected an anti-PSCA mAb cocktail. Tumor growth was monitored with caliper measurements. The control group included 6 mice (mice #1-6) and the 1G8 group included 7 mice (mice #7-13). The results show that the mice treated with the 1G8 antibody exhibited significant inhibition of tumor cell growth (Figure 54, lower panel) compared with the mice in the control group (Figure 54, upper panel). Thus, treatment with 1G8 alone inhibits the growth of tumor cells expressing PSCA.

7. Exhibits 2 and 3 depicts experimental results showing anti-PSCA monoclonal antibodies of different isotypes, each administered alone to animals that have been injected with tumor cells expressing PSCA, inhibit the growth of the PSCA-expressing tumor cells (Figure 55 A and B, respectively).

8. Exhibit 2 shows the results of mice injected with LAPC-9 and treated with mouse IgG, or 2A2, an IgG2a isotype, anti-PSCA monoclonal antibody (Figure 55A). The mice represented in Figure 55A were treated with either mouse IgG control (♦; diamonds), or with 2A2 (■; square). Tumor growth was monitored with caliper measurements. The results show that the mice treated with the 2A2 antibody exhibited significant inhibition of tumor cell growth (Figure 55A; ■) compared with the mice in the control group (Figure 55A; ♦). Tumor incidence was 6/6 mice in the mouse IgG control group, versus 2/7 for the 2A2-treated group. In the IgG control group, 4 out of the 6 mice had developed tumors by day 21. Thus, treatment with 2A2 alone inhibits the growth of tumor cells expressing PSCA.

9. Exhibit 3 shows the results of mice injected with LAPC-9 and treated with mouse IgG, or 2H9, an IgG1 isotype, anti-PSCA monoclonal antibody. The mice represented in Figure 55B were treated with either mouse IgG control (♦; diamonds), or with 2H9 (■; square). Tumor growth was monitored with caliper measurements. The control group included 6 mice and the 2H9 group included 7 mice. The results show that the mice treated with the

2H9 antibody exhibited significant inhibition of tumor cell growth compared with the mice in the control group. Tumor incidence was 6/6 mice in the mouse IgG control group, compared to 1/7 for the 2H9-treated group. In the 2H9-treated group the single tumor present appeared at day 21. In the mouse IgG control group, 4/6 of the mice had developed tumors by day 21. Thus, treatment with 2H9 alone inhibits the growth of tumor cells expressing PSCA.

10. Exhibit 4 depicts experimental results showing that administering an anti-PSCA antibody, to animals bearing established tumor cells, which express PSCA, inhibits the growth of the PSCA-expressing tumor cells (Figure 57). Exhibit 4 shows the results of mice bearing established LAPC-9 tumors (e.g., approximately 100 mm^3) treated with mouse IgG (♦; diamonds), or 3C5 (■; square) which is an IgG2a isotype, anti-PSCA monoclonal antibody. Tumor growth was monitored with caliper measurements. The results indicate that the 3C5 mAb inhibits the growth of established LAPC-9 prostate tumors. Some of the mice in the 3C5-treated group exhibited tumor regression up to 50% of the initial, pre-treatment size. Thus, treatment with 3C5 alone inhibits the growth of established tumor cells expressing PSCA.

11. Exhibit 5 depicts experimental results showing that the PSCA monoclonal antibodies exert tumor growth inhibition specifically through the PSCA protein on a PSCA-expressing cell (Figure 65). Exhibit 5 shows the results of mice injected with LAPC-9 cells which express PSCA (upper panel, Figure 65), or PC-3 which do not express PSCA (lower panel, Figure 65), and treated with mouse IgG or 1G8. Tumor growth was monitored with caliper measurements. The results show that the mice bearing LAPC-9 (Figure 65, upper panel; ♦) or PC-3 tumors (Figure 65, lower panel; ♦) and treated with mouse IgG exhibited significant tumor growth over a 40-day period. The mice bearing PC-3 tumors and treated with 1G8 also exhibited significant tumor growth (Figure 64, lower panel; ■). In contrast, the mice bearing LAPC-9 tumors and treated with 1G8

exhibited inhibited tumor growth. Thus, anti-PSCA antibodies, such as 1G8, inhibit tumor growth specifically through the PSCA protein on a PSCA-expressing cell.

12. Exhibit 6 depicts experimental results showing that administering an anti-PSCA antibody to animals bearing established orthotopic tumor cells which express PSCA inhibits the level of increase of serum PSA and inhibits the growth of the PSCA-expressing tumor cells (Figure 66A and B). The serum level of PSA was used to track the growth of the tumors, since the serum PSA level correlated well with the tumor size. The mice were segregated into two treatment groups, based on the levels of serum PSA. The group having low levels of PSA is depicted in Figure 66A and the group having moderate levels of PSA is depicted in Figure 66B. Exhibit 6 shows the results of mice bearing established orthotopic LAPC-9 tumors treated with phosphate buffer saline (PBS; ♦; diamonds), or 1G8 (■; square) which is an IgG1 isotype, anti-PSCA monoclonal antibody. The mice treated with 1G8, exhibited a reduction in the rate of increase in serum PSA levels (Figure 66A and B), compared with the mice treated with PBS. Thus, treatment with 1G8 alone reduces the rate of increase in serum PSA levels, which correlates with inhibiting the growth of tumors expressing PSCA.

13. Exhibit 7 depicts experimental results showing that administering an anti-PSCA antibody, to animals bearing established orthotopic tumor cells which express PSCA, increased the survival of the tumor-bearing mice (Figure 67A and B). The mice described in Exhibit 7 (e.g., treated with PBS or 1G8) were permitted to live, to determine the length of survival time. The mice treated with PBS began to die within 5-6 weeks post-injection, due to local tumor growth and metastasis. In contrast, the mice treated with 1G8 antibody exhibited a prolonged life. The mice having lower serum PSA levels and treated with 1G8 (Figure 67A) exhibited the greatest increase in survival. The inhibition of tumor growth correlated with prolonged life. Thus, treatment with 1G8 increased survival time, by inhibiting tumor growth.

14. Exhibit 8 depicts experimental results showing that administering an anti-PSCA antibody to animals bearing established orthotopic tumor cells which express PSCA inhibits the level of increase of serum PSA and inhibits the growth of the PSCA-expressing tumor cells (Figure 68A and B). The serum level of PSA was used to track the growth of the tumors, since the serum PSA level correlated well with the tumor size. The mice were segregated into two treatment groups, based on the levels of serum PSA. The group having low levels of PSA is depicted in Figure 68A and the group having moderate levels of PSA is depicted in Figure 68B. Exhibit 8 shows the results of mice bearing established orthotopic LAPC-9 tumors treated with phosphate buffer saline (PBS; ♦; diamonds), or 3C5 (■; square) which is an IgG2a isotype, anti-PSCA monoclonal antibody. The mice treated with 3C5, exhibited a reduction in the rate of increase in serum PSA levels (Figure 68A and B), compared with the mice treated with PBS. Thus, treatment with 3C5 alone reduces the rate of increase in serum PSA levels, which correlates with inhibits growth of tumors expressing PSCA.

15. Exhibit 9 depicts experimental results showing that administering an anti-PSCA antibody, to animals bearing established orthotopic tumor cells which express PSCA, increased the survival of the tumor-bearing mice (Figure 69A and B). The mice described in Exhibit 9 (e.g., treated with PBS or 3C5) were permitted to live, to determine the length of survival time. The mice treated with PBS began to die within 5-6 weeks post-injection, due to local tumor growth and metastasis. In contrast, the mice treated with 3C5 antibody exhibited a prolonged life. The mice having lower serum PSA levels and treated with 3C5 (Figure 69A) exhibited the greatest increase in survival. The inhibition of tumor growth correlated with prolonged life. Thus, treatment with 3C5 increased survival time, by inhibiting tumor growth.

16. Exhibit 10 depicts experimental results showing that administering an anti-PSCA antibody alone or in combination with doxorubicin to animals bearing established tumor cells which express PSCA inhibits the growth of the PSCA-expressing tumor cells (Figure 70). Exhibit 10 shows the results of mice bearing established PC3-PSCA tumors (e.g., approximately 100-200 mm^3) treated with PBS, 1G8 alone, doxorubicin alone, or a combination of 1G8 and doxorubicin. The PC3-PSCA cells were derived by retroviral gene transfer of the PSCA gene into PC3 cells which do not express PSCA and are androgen-independent. Tumor growth was monitored with caliper measurements. The mice treated with doxorubicin alone exhibited a slightly lower tumor growth rate, compared to mice treated with PBS. In contrast, mice treated with 1G8 antibody alone exhibited a greater reduction in tumor growth rate, compared to the mice treated with PBS. The mice treated with the combination of 1G8 and doxorubicin exhibited a slightly greater reduction in tumor growth rate, compared to the mice treated with PBS, 1G8 alone or doxorubicin alone. Thus, treatment with 1G8 in combination with doxorubicin inhibits the growth of established, androgen-independent tumor cells expressing PSCA.

17. Exhibit 11 depicts experimental results showing that an anti-PSCA monoclonal antibody, administered to a tumor-bearing animal, selectively targets tumor cells expressing PSCA (Figure 71). Exhibit 11 shows the results of immunohistochemistry analyses of tumor explants from the mice described in Exhibit 4. These mice bear established LAPC-9 tumors (e.g., approximately 100 mm^3) and were treated with mouse IgG, or 3C5 which is an IgG2a isotype, anti-PSCA monoclonal antibody. The tumor explants from the mice in both treated groups were analyzed by immunohistochemistry. The tumor explants were sliced and fixed, and probed with goat anti-mouse IgG to detect the presence of antibody. The results show that the 3C5 antibody was detected throughout the tumor explants and was localized specifically in the tumor explants expressing PSCA but not in the cells surrounding the tumor. In contrast, no antibody was detected in the tumor explants from

the mice treated with IgG. Thus, the administered 3C5 monoclonal antibody specifically localized to tumor cells expressing PSCA.

18. Exhibit 12 depicts experimental results showing that an anti-PSCA monoclonal antibody, administered to a tumor-bearing animal, selectively targets tumor cells expressing PSCA (Figure 72). Exhibit 12 shows the results of a Western blot analysis of tumor lysates from the mice described in Exhibit 4. These mice bear established LAPC-9 tumors (e.g., approximately 100 mm^3) and were treated with mouse IgG, or 3C5 which is an IgG2a isotype, anti-PSCA monoclonal antibody. The tumor lysates from the mice in both treated groups were analyzed by Western blot analysis. The tumor lysates were electrophoresed in a gel, transferred to a solid support, and the solid support was probed with goat anti-mouse IgG-HRP antibodies to detect the presence of antibody. The mouse IgG control antibody and 3C5 were also run on the gel as controls. The results show that the that IgG heavy and light chains were readily detected in tumor lysates from the 3C5-treated mice, but not in the mouse IgG control treated mice. Thus, the administered 3C5 monoclonal antibody specifically localized to tumor cells expressing PSCA.

19. Exhibit 13 depicts experimental results showing that an anti-PSCA monoclonal antibody, administered to a tumor-bearing animal, selectively targets tumor cells expressing PSCA (Figure 73). Exhibit 13 shows the results of a Western blot analysis of tumor lysates from mice treated with 1G8 or mouse IgG. These mice bear established LAPC-9 tumors (e.g., approximately 100 mm^3) and were treated with mouse IgG, or 1G8 which is an IgG1 isotype, anti-PSCA monoclonal antibody. The tumor lysates from the mice in both treated groups were analyzed by Western blot analysis. The tumor lysates were electrophoresed in a gel, transferred to a solid support, and the solid support was probed with goat anti-mouse IgG-HRP antibodies to detect the presence of antibody. The mouse IgG control antibody and 1G8 were also run on the gel as controls. The results show that the that IgG heavy and light chains were readily detected in tumor lysates from the 1G8-

treated mice, but not in the mouse IgG control treated mice. Thus, the administered 1G8 monoclonal antibody specifically localized to tumor cells expressing PSCA.

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issuing thereon.

8/30/06
DATE

Robert E. Reiter

DATE

Owen N. Witte